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Circular No. 576

December 1940 • Washington, D. C.

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The Use of Chlorine Gas as a Seed Disinfectant

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INTRODUCTION

Chlorine compounds, especially hypochlorites, have been used as disinfectants for more than a century. At present they are much used for sterilization purposes in city water reservoirs, swimming pools, dairy plants, and even in medical practice.

Little mention, however, of the direct use of chlorine gas as a fungicide is found in the literature. In the spring of 1937 the attention of the authors was called to the commercial use of chlorine gas for treating seed grain and to the application for a patent on the process. It was claimed that this form of treatment was superior to dust and liquid treatments in the following respects: (1) It was much cheaper; (2) it killed insects as well as fungi infesting the grain; (3) it improved germination, radication, and stand; (4) it did not render the seed unfit for feed or food; and (5) it eliminated objectionable odors and improved the appearance of the grain. In view of these claims and of inquiries regarding this form of treatment, it was deemed urgent to determine the effectiveness and suitability of chlorine gas for seed treatment, and the experiments here described were conducted. These experiments were of two types: (1) Laboratory treatments, in which the amounts and concentrations of gas used were accurately controlled; and (2) commercial treatments, in which the chlorine gas was applied by means of an outfit designed and used commercially for that purpose.

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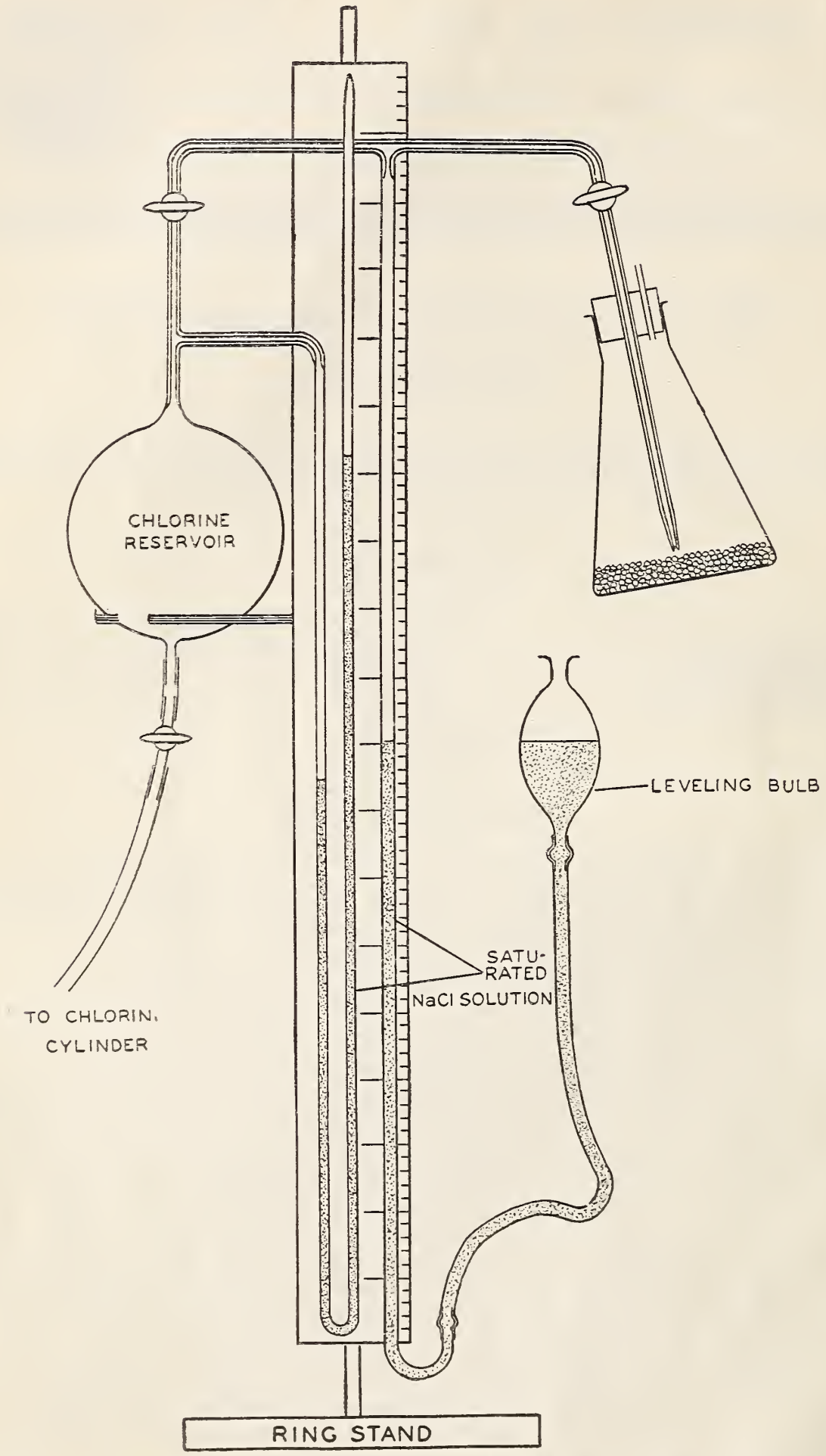

FIGURE 1.—Apparatus for delivering accurately measured volumes of chlorine gas.

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MATERIALS AND METHODS

The chlorine gas used was taken from a 1-pound drum of liquid chlorine. The gas was led into a specially constructed burette (fig. 1) from which accurately measured quantities were forced, under hydrostatic pressure of 1 atmosphere, into the flasks containing the seed to be treated. A concentrated sodium chloride solution saturated with chlorine gas was used for forcing the gas out of the burette. In this circular all concentrations of gas are expressed in terms of relative volume of gas and air.

In studying the lethal effect of chlorine gas on smut spores, the authors used mostly spores of covered smut of barley (*Ustilago hordei* (Pers.) Kell. and Sw.) and covered kernel smut of sorghum (*Sphacelotheca sorghi* (Lk.) Clint.) because of the readiness with which the spores of these smuts germinate on agar. Spores of bunt (*Tilletia* spp.) were found less suitable for this purpose because of their time and temperature requirements for germination. The spore-germination tests were made by placing a few smutted treated seeds on plates of plain water agar, shaking the plates slightly to scatter some of the spores on the agar, and then removing the seeds. The viable spores of barley smut and sorghum smut germinated in about 8 hours at 25° C.

In studies on the effect of chlorine gas on germination of cereal seeds, Purplestraw wheat, Odessa barley, Fulghum and Victory oats, and Chiltex sorgo were used. Germination tests of treated and nontreated seed were made on wet paper towels and in small tins of sterilized soil kept at a temperature of 20° C. Additional data on germination were obtained from seed sown for determining smut control in greenhouse and outdoor beds.

In laboratory, greenhouse, and field studies on the efficiency of chlorine gas in smut control, the following lots of cereal seeds were used: Purplestraw and Baart wheat, artificially dusted with bunt spores at a spore dosage of 1 to 100, and five lots of wheat from different farms, naturally smutted and commercially treated with chlorine; Odessa and Wisconsin Winter barley; Fulghum, Victory, and Lee oats; Sharon kafir; Scarborough broomcorn; and Leoti sorgo. With the exception of one lot of Lee oats, dusted with spores of oat covered smut (*Ustilago levis* (Kell. and Sw.) Magn.), the barley and oats were inoculated with spores of the covered smuts of oats and barley, respectively, by the spore suspension vacuum method described previously.[1] The three lots of sorghum seed were dusted with spores of covered kernel smut at a spore dosage of 1 to 100.

Smut data in the greenhouse were based on plant counts and those taken outdoors were based on head counts.

[1] LEUKEL, R. W. SEED TREATMENT EXPERIMENTS WITH OATS NATURALLY AND ARTIFICALLY INOCULATED WITH SMUTS. U. S. Dept. Agr. Tech. Bul. 568, 16 pp. 1937.

EXPERIMENTAL RESULTS

Laboratory Treatments

The lack of any available data on the effect of chlorine gas on cereal seeds and on the spores of cereal smuts made it necessary to try wide ranges of gas concentration and duration of exposure in order to find suitable combinations of these two factors that would kill the smut spores without injuring the seeds. Accordingly, in the first experiment small lots of wheat, barley, oats, and sorghum were placed in separate 500-cc. flasks, and the air in each flask was replaced with pure chlorine. Seeds removed from each flask for germination tests after 2 hours and after 20 hours were found to have been greatly impaired in viability. The wheat was more severely injured than were the barley, oats, and sorghum.

In a second series 140-cc. flasks, each containing 50 gm. of seed, were charged with different amounts of chlorine so that the following percentages by volume of gas in air resulted: 0.6, 1.2, 1.5, 2.0, 3.0, and 6.0. The volume of pure chlorine gas in the flasks ranged from 1 to 10 percent of the net volume of grain.[2] Germination tests made after 2 hours' and again after 20 hours' exposure showed that the viability of the seed had not been impaired.

In another series, flasks of 125-cc. capacity were used with 25 gm. of seed in each. The volume of gas introduced into each flask ranged from 10 to 33 percent of the total volume of the flask and from 20 to 90 percent of the net volume of the seed. Germination tests of the seed made after an exposure of 2 hours and after one of 20 hours showed, with few exceptions, moderate to severe reductions in the percentages of germination. Wheat was injured the most and Fulghum oats the least. The germination results from these three experiments are shown in table 1.

Table 1.—*Effect of exposure to chlorine gas on germination of cereal seeds*

Concentration of chlorine gas in air [1] (percent)	Germination of seed after exposure to chlorine gas for—									
	2 hours					20 hours				
	Wheat	Barley	Fulghum oats	Victory oats	Sorgo	Wheat	Barley	Fulghum oats	Victory oats	Sorgo
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0.6	96	98	96	96	91	90	97	94	99	92
1.2	97	97	94	95	92	96	98	94	97	90
1.5	95	100	95	98	88	97	99	96	98	88
2.0	93	98	93	97	89	96	99	93	98	91
3.0	94	97	97	98	90	95	97	95	97	88
6.0	96	97	95	96	90	94	99	95	97	91
10 to 13	79	87	91	95	95	46	73	83	93	87
15 to 20	53	71	89	85	82	52	75	91	61	77
22 to 27	45	76	78	57	60	46	70	79	48	70
26 to 33	45	73	66	55	87	46	74	70	60	83
100	21	72	60	40	50	2	33	34	29	35
None (control)	96	98	95	97	89	95	98	93	98	90

[1] Expressed as percent by volume of chlorine in air.

[2] During these first tests the concentration of the gas was based wholly on the relative volume of gas and air in the flask, and the relative volume of gas and seed was not taken into consideration. Subsequent experiments showed this relation to be important.

The data in table 1 show that under the conditions of the experiments the seeds of these cereals may be injured by exposure for 2 hours to a concentration of chlorine gas of 10 percent or more.

To determine the lowest concentration of chlorine gas that would be lethal to the spores of covered smut of barley and covered kernel smut of sorghum, concentrations of 0.5 to 25 percent of chlorine gas were used. Only 10 gm. of smutted seed were placed in each flask so that the actual volume of the gas used ranged from 30 to 180 percent of the volume of the seed in the flasks. The results from germination tests of the spores made after 2 hours and again after 20 hours are shown in table 2.

TABLE 2.—*Germination of spores of Ustilago hordei and Sphacelotheca sorghi as affected by exposure to different concentrations of chlorine gas*

Concentration of chlorine gas in air (percent)	Germination of spores after exposure to chlorine gas for—			
	2 hours		20 hours	
	U. hordei	*S. sorghi*	*U. hordei*	*S. hordei*
	Percent	*Percent*	*Percent*	*Percent*
None (check)	60	80	60	80
0.5	5	2	(1)	(1)
1.0	2	(1)	0	(1)
2.5	(1)	(1)	0	(1)
5.0	0	(1)	0	(1)
7.5	0	(1)	0	0
10.0	0	0	0	0
15.0	0	0	0	0
20.0	0	0	0	0
25.0	0	0	0	0

[1] Trace (less than 1 percent).

Inasmuch as exposure to 0.5-percent chlorine gas for 2 hours reduced the germination of spores of barley and sorghum smuts to 5 and 2 percent, respectively, it was thought that perhaps a slightly higher concentration for a shorter period would effect smut control without seed injury. Accordingly, different lots of 100 seeds of wheat and of Chiltex sorghum, dusted with spores of barley covered smut and sorghum covered kernel smut, respectively, were exposed to concentrations of chlorine gas ranging from 3 to 9 percent by volume for periods of one-fourth, one-half, 1, 2, and 20 hours. The resulting data on the germination of seed and spores are shown in table 3.

TABLE 3.—*Effect of exposure to different concentrations of chlorine gas for different periods of time on the germination of wheat and sorghum seed and spores of Ustilago hordei and Sphacelotheca sorghi*

Duration of exposure to gas (hours)	Concentration of chlorine gas in air	Germination of—			
		Seed		Smut spores	
		Wheat	Sorghum	*U. hordei*	*S. sorghi*
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
0		94	93	45	60
¼	3	94	94	40	55
	5	94	94	40	50
	7	91	92	40	40
	9	92	90	30	30
½	3	94	92	35	30
	5	92	92	30	2
	7	86	83	25	1
	9	74	85	5	(1)
1	3	91	93	5	(1)
	5	84	87	2	(1)
	7	58	89	(1)	(1)
	9	49	81	(1)	0
2	3	89	90	1	(1)
	5	61	90	(1)	(1)
	7	49	86	(1)	(1)
	9	43	79	(1)	0
20	3	43	74	0	(1)
	5	53	60	0	(1)
	7	42	58	0	(1)
	9	42	66	0	0

[1] Trace (less than 1 percent).

Although exposure to 9-percent chlorine gas for 15 minutes caused no significant reduction in the germination of seeds of wheat and sorghum it also failed to reduce the germination of the smut spores more than 50 percent. Exposure to 7- and 9-percent chlorine for one-half hour caused some indication of seed injury without entirely inhibiting smut spore germination.

The data in table 3 seem to indicate that the "margin of safety" is rather narrow; that is to say, the combination of chlorine gas concentration and the period of exposure necessary to kill all the smut spores seems to be very near or even to overlap the point where seed injury occurs.

It also seems evident from the data in table 3 that low concentrations of gas for a relatively short period of time, i. e., 15 minutes or less, are not effective in killing smut spores, and that from one-half to 1 hour is necessary for smut control with concentrations as low as 9 percent. This treatment, however, not only approaches the point where seed injury occurs, but is objectionable from the practicable standpoint of time required, because it could not be adapted to the types of continuous seed-treatment outfits now commonly used. Its application to large quantities of seed would require gastight containers of considerable capacity and would offer other difficulties.

It was decided to try higher concentrations of gas and still shorter periods of exposure in order to find, if possible, a combination that would be practicable and effective for seed treatment. Chlorine concentrations of 20, 33, 57, and 77 percent were used for 1, 2, and 4 minutes in 50-cc. flasks, each containing 200 smutted seeds of either wheat or sorghum. The germination of the smut spores was not greatly affected except at the two highest concentrations and at the longest period of exposure (4 minutes). However, this treatment

also was deleterious to the germination of the wheat, although the viability of the sorghum seemed not to have been affected. Concentrations of 50- and 100-percent chlorine gas for 5, 10, 15, and 20 minutes also were used. Germination tests again showed that seed injury began at a point where inhibition of smut spore germination was not complete. Even after exposure in an atmosphere of pure chlorine gas for 5 minutes, 1 percent of the barley smut spores and 4 percent of the sorghum smut spores germinated, and the germination of the wheat was reduced 53 percent. The germination of sorghum seed apparently was not affected by this short exposure. These results are presented in table 4.

TABLE 4.—*Effect of exposure to relatively high concentrations of chlorine gas for short periods of time on the germination of seeds of wheat and sorghum and spores of Ustilago hordei and Sphacelotheca sorghi*

Concentration of chlorine gas in air (percent)	Duration of exposure	Germination of —			
		Seeds of—		Spores of—	
		Wheat	Sorghum	*U. hordei*	*S. sorghi*
	Minutes	*Percent*	*Percent*	*Percent*	*Percent*
0		87	82	60	63
20	1	90	84	48	65
	2	87	88	45	65
	4	86	87	40	65
33	1	88	85	50	63
	2	82	87	45	65
	4	65	78	40	60
57	1	86	80	50	65
	2	77	81	40	60
	4	60	81	10	30
77	1	76	86	40	42
	2	49	80	15	35
	4	48	80	(1)	7
50	5	42	83	1	6
	10	41	74	(1)	1
	15	36	72	(1)	1
	20	40	69	0	(1)
100	5	41	85	1	4
	10	47	80	0	(1)
	15	40	77	0	(1)
	20	43	70	0	0
0		90	92	60	40

[1] Trace (less than 1 percent).

On the assumption that the gas absorbed by a larger bulk of treated seed possibly continues the disinfecting process after the grain is sacked, the following experiment was tried. Different amounts of chlorine gas were forced into a number of 550-cc. flasks each containing 250 cc. of grain infested with spores of *Ustilago hordei*, so that concentrations of 3-, 5-, and 7-percent chlorine gas resulted. These concentrations were maintained for periods ranging from 3 minutes to 2 hours, after which the contents of each flask were emptied into a tightly woven cloth sack, with the mouth of the flask remaining in the sack for several hours. Spores were then plated at 2-hour intervals for 24 hours. In no case was there a sufficient reduction in spore germination to suggest smut control. Even in the flasks that had remained stoppered for 2 hours the inhibition of spore germination was inadequate for satisfactory smut control, although in a previous experiment (table 2) a concentration of only 2.5 percent for 2 hours had reduced spore germination to a trace.

The contradictory results obtained with combinations of gas concentrations and periods of exposure that had inhibited spore germination in previous experiments indicated that some factor had been overlooked in this experiment. A study of the data from the previous experiments seemed to show that when a relatively small quantity of smutty seed had been treated for a given time in a relatively large flask the seed had been more easily injured and the spores more readily killed in a given concentration of gas than when a much larger quantity of seed had been used in a flask of the same size containing the same concentration of gas. To study this relation, different volumes of seed were placed in flasks of different sizes but containing the same concentrations of gas. In stating the volume of the seed, the net volume is meant, allowance being made for the voids between the seeds, which in wheat is about 40 percent of the total space occupied. The volume of chlorine gas used in the different flasks ranged from 1.6 percent to 140 percent of the net volume of seed, but only from 1 to 7 percent of the volume of the air. As short periods of exposure had proved relatively ineffective, a 2-hour period was again used. The data on the germination of the wheat and of the barley smut spores are shown in table 5.

TABLE 5.—*Germination of seed of wheat and of spores of Ustilago hordei as affected by exposure for 2 hours to 4 different concentrations of chlorine gas with a concurrent range in the relative volume of gas and seed at each concentration*

Volume of—			Percentage of chlorine based on relative volume of gas and—		Germination of—	
Flask	Seed	Gas	Air	Seed	Seed of wheat	Spores of *U. hordei*
Cc.	*Cc.*	*Cc.*	*Percent*	*Percent*	*Percent*	*Percent*
40	25.0	0.4	1	1.6	96	22
120	37.5	1.2	1	3.2	93	15
240	25.0	2.4	1	9.6	95	13
250	12.5	2.5	1	20.0	94	0
40	25.0	1.2	3	4.8	96	17
120	37.5	3.6	3	9.6	91	13
240	25.0	7.2	3	28.8	94	0
250	12.5	7.5	3	60.0	84	0
40	25.0	2.0	5	8.0	95	12
120	37.5	6.0	5	16.0	97	(1)
240	25.0	12.0	5	48.0	85	0
250	12.5	12.5	5	100.0	70	0
40	25.0	2.8	7	11.2	93	10
120	37.5	8.4	7	22.4	90	0
240	25.0	16.8	7	67.2	67	0
250	12.5	17.5	7	140.0	63	0
-----	----	----	0	0	96	40

(1) Trace (less than 1 percent).

The results suggest that for an exposure of 2 hours to concentrations of chlorine gas ranging from 1 to 7 percent, the actual volume of the gas used should not be less than 20 percent or probably not more than 40 percent of the net volume of the seed being treated if elimination of externally borne smut spores is to be obtained without injury to the seed. For example, when the air in the flask contained 7 percent of chlorine gas but the actual volume of gas used to produce this concentration was only 11.2 percent of the volume of the seed, spore germination was reduced only 75 percent; yet, when the air contained only

1 percent of chlorine gas and the volume of chlorine was 20 percent of the volume of the seed, complete killing of the spores followed after an exposure of 2 hours. Nugent and Cook [3] report similar results in the treatment of kale seed with chloropicrin gas. They used concentrations of 0.088 to 0.35 percent in air for 24 hours, and found that higher concentrations of gas were required for thorough disinfection when the chamber was nearly full of seed than when only one-fourth filled, indicating that some of the gas is absorbed by the seed.

With these limitations of the chlorine treatment in mind, experiments were conducted in the field and in the greenhouse to test the efficacy of chlorine gas in actual smut control. Separate portions of smutty seed of wheat, barley, and oats were exposed for 2 hours in flasks to concentrations of chlorine gas ranging from 3.3 to 6.6 percent. Separate portions of each seed lot also were treated in bulk in a 20-gallon rotary-drum treater to parallel, to some extent, the conditions under which seed presumably might be treated commercially. A quantity of chlorine gas equal to 9 percent of the volume of the air and 40 percent of that of the seed in the treater was forced into the treater, which was then sealed and the grain agitated for 4 minutes. The grain was allowed to remain in the closed treater for 2 hours and then sacked. Other portions of smutty seed from the same lots were treated with copper carbonate, New Improved Ceresan, or formaldehyde for comparison. Some seeds of each portion were planted in small tins of sterilized soil and the seedlings allowed to emerge under conditions optimum for the development of the different smuts. They were then transplanted to beds in the greenhouse or outdoors. Other seeds of each treated portion were sown directly outdoors. The data from the above experiment are shown in table 6.

[3] NUGENT, T. J., and COOK, HAROLD T. CHLOROPICRIN AS A SEED DISINFECTANT FOR CONTROL OF BLACK ROT OF KALE. (Abstract.) Phytopath. 29: 19. 1939.

TABLE 6.—*Effect of 2 hours' exposure to different concentrations of chlorine gas on the germination of seed and smut spores and on smut infection compared with the effects of other treatments*

Crop	Fungicide used	Concentration of gas based on relative volume of gas and—		Germination of—			Smut infection in series [1]—				Average infection
				Seed		Smut spores	1	2	3	4	
		Air	Seed	Indoors	Outdoors						
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Baart wheat	None			84	78	50	93	96	28		72
	Chlorine	4.2	15	84	81	4	9	12	1		7
	do	3.7	22	80	82	1	3	3	0		2
	do	3.4	30	81	75	1	4	6	0		3
	do.[2]	9.0	40	84	77	([3])	4	3	0		2
	Copper carbonate [4]			85	80	0	1	1	0		1
	New Improved Ceresan [5]			89	82	0	0	0	0		0
Odessa barley	None			82	81	50	93	80	31	20	56
	Chlorine	5.0	20			5				17	
	do	3.3	30	76	84	7	93	87	28	17	56
	do	4.4	40	80	90	([3])	86	81	29	8	51
	do.[2]	9.0	40	76	87	([3])	93	90	36	15	59
	New Improved Ceresan			91	94	0	0	0	0	0	0
Victory oats	None			64	69		100	98	97	73	92
	Chlorine	3.7	20							63	
	do	5.5	30	83	74		90	91	41	42	66
	do	3.4	40	85	77		76	73	41	26	54
	do.[2]	9.0	40	71	63		96	97	59	40	73
	New Improved Ceresan			90	83		3	2	3	2	3
	Formaldehyde [6]				64				6	5	6
Fulghum oats	None			50	30		63	91	82	40	69
	Chlorine	6.6	20							53	
	do	4.3	30	62	52		50	85	67	30	58
	do	5.7	40	72	68		50	87	54	28	55
	do.[2]	9.0	40	54	47		60	90	80	35	66
	New Improved Ceresan			84	65		2	4	4	2	3

[1] Series 1, emerged at optimum temperature and transplanted in greenhouse. Series 2, emerged at optimum temperature and transplanted outdoors. Series 3, seed sown in outdoor beds. Series 4, seed sown in field plots.
[2] Exposed to chlorine in ordinary churn treater.
[3] Trace (less than 1 percent).
[4] Applied at rate of 2 ounces per bushel.
[5] Applied at rate of ½ ounce per bushel.
[6] Seed dipped in 1:320 solution, drained, covered 2 hours, and dried.

Bunt in wheat was fairly well controlled by chlorine, considering the heavy infection in wheat from untreated seed in two of the series, but copper carbonate and New Improved Ceresan were superior to chlorine in this respect.

Chlorine gas failed to effect satisfactory control of covered smuts in barley and oats despite the fact that surface-borne spores of barley smut had been killed by chlorine gas in some of the preliminary tests. The smut spores beneath the glumes of the barley and oats evidently were protected from the chlorine gas and thus escaped its fungicidal action. New Improved Ceresan, on the other hand, completely eliminated barley smut and effected 96 to 97 percent control of the oats smut. Formaldehyde, where used, was less effective than New Improved Ceresan and also impaired germination.

In a separate experiment, three lots of sorghum seed were exposed to a 10-percent concentration of chlorine gas for 1 hour. The volume of gas in each flask was equal to 40 percent of the volume of the seed used. The seed was planted in the field along with untreated smutty seed for comparison. The percentages of smutted heads grown from untreated seed of Sharon kafir, Scarborough broomcorn, and Leoti sorgo were 76, 61, and 57, respectively. Smut was completely eliminated in the kafir and broomcorn and was reduced to 1.4 percent in the sorgo. The field germination of the treated seed was 36, 59, and 37 percent for the kafir, broomcorn, and sorgo, respectively, compared with 45, 65, and 38 percent, respectively, for untreated seed. The slightly superior germination of the untreated seed suggests the possibility of some injury to the seed following treatment with chlorine.

Commercial Treatments

Although chlorine gas under certain conditions during the 1937–38 season showed some promise as a seed fungicide so far as surface-borne smut spores are concerned, its practical application seemed to offer some difficulties. According to the results obtained in these experiments, short-time exposures were not entirely effective in killing smut spores, regardless of the concentration of chlorine gas used. Furthermore, any combination of short-time exposure with high gas concentration that approached fungicidal effectiveness injured seed germination. Yet observations made on the custom treatment of seed grain with chlorine gas, as carried out in the commercial treater designed for that purpose, showed that the grain was being run through the treater in about 3 minutes, and that during that time it was being exposed to what appeared to be a relatively low concentration of chlorine gas, as determined by a flowmeter inserted in the gas line. It was claimed that this treatment controlled stinking smut in wheat and smuts and other diseases in other cereals, and, in addition, killed weevils and other insects in the grain. Arrangements were made, therefore, to run several lots of smutty seed through this commercial treater to determine to what extent, if any, smut control could be obtained by this method of treatment.

A bushel of Lee oats was thoroughly dusted with spores of oat covered smut. Half of this seed was then subjected to further inoculation by immersion in a spore suspension under vacuum. A quantity of Wisconsin Winter barley was similarly inoculated by the vacuum method with spores of barley covered smut.

Several bushels of Purplestraw wheat were dusted with bunt spores at a spore dosage of 1 to 100. The above seed lots were transported in separate sealed metal cans to the commercial plant where they were to be treated with chlorine gas. In addition to these seed lots, five lots of smutty wheat from different farms in that locality were obtained from a nearby mill.[4]

The two lots of oats and one of barley were run through the commercial treater twice. The different lots of wheat were divided into two portions. One portion of each lot was run through the cleaning machinery with which the plant was equipped. The other portion was not cleaned.

In treating the wheat, one-third of each cleaned and uncleaned portion was run through the treater once and one-third was run through twice. The other third remained untreated.

The time required for a lot of grain to pass through the treater once was observed to be about 3 minutes. By means of an aspirator, accurately measured quantities of the air and chlorine mixture were drawn from the interior of the treating drum while it was in operation. These were taken to the laboratory and the percentage of chlorine gas in the mixture was determined. The results of 10 determinations showed that the concentration of gas inside the treating drum ranged from 0.2 to 0.46 percent, the average being 0.35 percent.

The treated and untreated portions of cleaned and uncleaned seed were transported to the Arlington Experiment Farm, Arlington, Va., in separate sealed containers, every precaution being taken to prevent recontamination of the treated seed. The seed was sown with a drill in 132-foot rows on the Arlington Farm, similar precautions being taken. Separate portions of each lot of inoculated seed treated with New Improved Ceresan were sown for comparison. Formaldehyde also was used for barley and oats. Separate lots of the smutty Purplestraw wheat were treated also with copper carbonate and basic copper sulfate, and sown in a separate series. Data on germination were obtained from a separate plot planted by hand. The percentages of smutted heads from the different seed lots were determined at the proper time by counting the total and smutted heads in 10 representative parts of each drill row. These data are presented in tables 7 and 8.

[4] Grateful acknowledgement is made of the assistance of A. G. Johnson, of the Division of Cereal Crops and Diseases, who kindly carried out part of the work.

TABLE 7.—*Emergence and smut infection in Lee oats and Wisconsin Winter barley as affected by inoculation* [1] *and treatment of seed sown in 1/80-acre plots at the Arlington Experiment Farm, 1938–39*

Crop	Form of inoculum used	Treatment of seed	Emergence	Smut infection
			Percent	*Percent*
Lee oats	None	None	90	9.0
		do	86	37.0
	Dry spores applied to seed	Chlorine gas [2]	90	32.0
		New Improved Ceresan [3]	92	0
		Formaldehyde [4]	80	0
	Spore suspension in vacuum	None	78	64.8
		Chlorine gas [2]	70	66.0
		New Improved Ceresan [3]	83	7.9
		Formaldehyde [4]	51	1.3
Wisconsin Winter barley	None	None	92	0
	Spore suspension in vacuum	do	90	36.7
		Chlorine gas [2]	90	34.3
		New Improved Ceresan	92	0
		Formaldehyde [5]	40	0

[1] The oats and barley were inoculated with spores of *Ustilago levis* and *Ustilago hordei*, respectively.
[2] Applied in a commercial treater at a gas concentration of approximately 0.35 percent over a period of 6 minutes.
[3] Applied at ½ ounce per bushel.
[4] Immersed in 1:320 solution until wet, drained, covered 3 hours, and dried.
[5] Immersed in 1:240 solution 1 hour, drained, and dried.

TABLE 8.—*Emergence and bunt control in different lots of winter wheat (farm samples) as affected by cleaning the seed and by treating it with chlorine gas* [1] *or New Improved Ceresan*

Type of seed and lot	Emergence from seed—				Bunted heads from seed—			
	Not treated	Treated with—			Not treated	Treated with—		
		Chlorine gas		New Improved Ceresan		Chlorine gas		New Improved Ceresan
		Once	Twice			Once	Twice	
Uncleaned seed:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	83	82	79	94	50	40	35	[2]
2	54	51	48	80	45	45	38	0
3	88	78	83	95	52	50	45	0
4	52	57	60	73	45	40	46	0
5	76	75	78	78	48	41	34	0
6 [3]	84	81	93	90	96	90	84	[2]
Average	73	71	74	85	56	51	47	[2]
Cleaned seed:								
1	85	83	86	93	40	22	21	[2]
2	67	74	65	76	41	20	20	0
3	82	86	90	95	43	30	22	0
4	60	53	67	73	40	24	19	0
5	70	73	72	88	42	38	30	0
6 [3]	89	80	88	94	94	88	80	[2]
Average	76	75	78	87	50	37	32	[2]

[1] The chlorine gas treatment was carried out in a commercial treater, constructed and commercially operated for chlorine treatment of seed. The gas concentration in the drum of the treater averaged 0.35 percent, and the duration of exposure was about 3 minutes.
[2] Trace (less than 1 percent).
[3] Purplestraw wheat, artificially inoculated.

The failure of the commercially applied chlorine gas treatment to affect, significantly, either the germination or the incidence of smut in oats or barley was to be expected in the light of previous field tests with seed treated in the laboratory with higher concentrations of gas and longer periods of exposure. New Improved Ceresan effectively

eliminated barley covered smut and also oat smut except where the vacuum method of inoculation had been used. Formaldehyde, although controlling barley and oat smuts satisfactorily, injured the germination of the seed.

The commercially applied chlorine gas treatment also failed to improve emergence and stand or to effectively control bunt in any of the different lots of wheat. Cleaning the wheat in some cases seemed to cause an increase in the percentage of germination and also some decrease in the percentage of bunted heads, but most of these differences were not great enough to appear significant. New Improved Ceresan caused considerable increases in the percentages of germination in some seed lots, the increases in uncleaned seed ranging from 2 to 26 and in cleaned seed from 5 to 18. Satisfactory control of bunt was obtained with New Improved Ceresan in all cases. Copper carbonate and basic copper sulfate reduced the percentage of bunt in Purplestraw from 95 percent to less than 1 percent.

Effect of Chlorine on Weevils

The possibilities of chlorine gas as a fumigant against weevils was investigated only in a preliminary way. Adults of the rice weevil (*Sitophilus oryza* L.), the granary weevil (*Sitophilus granarius* (L.)), and the confused flour beetle (*Tribolium confusum* Jacq. Duv.) were placed in Erlenmeyer flasks, about 50 insects being placed in each flask. The concentrations of chlorine used were 1, 2, 5, 10, 20, 50, and 100 percent, by volume, and the periods of exposure were 2, 5, 10, 20, 50 ,30, and 60 minutes. The different amounts of chlorine gas were measured into the respective flasks, which were then quickly sealed for the given periods, after which they were opened and the chlorine driven out by compressed air. A screen was placed over the mouth of each flask, and the insects were observed over a period of several days. A count was then made of the weevils in each flask showing any signs of life, and the percentages of weevils remaining alive in the different flasks are shown in table 9.

Table 9.—*Lethal effect of different concentrations of chlorine gas for different periods of time on weevils* [1] *in glass flasks*

Volume of—		Concentration of gas	Weevils alive [2] after exposure to chlorine for—					
Flask	Chlorine		2 minutes	5 minutes	10 minutes	20 minutes	30 minutes	60 minutes
Cc.	*Cc.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1,000	10.0	1	75	35	30	11	17	0
1,000	20.0	2	60	40	7	15	0	0
500	25.0	5	60	30	22	26	15	14
250	25.0	10	42	24	4	16	11	9
150	30.0	20	38	20	27	12	0	0
50	25.0	50	25	0	15	0	0	0
50	50.0	100	0	0	0	0	0	0

[1] About 50 adult weevils were in each flask. The confused flour beetles when included usually succumbed first.

[2] If a weevil showed any indication of life after 2 days it was recorded as alive.

The ability of the weevils to survive was governed largely, it seems, by their size and condition, as the larger and more active insects invariably were the ones to survive. The fact that the weevils in

the different flasks may have varied greatly in the above respects makes the data seem somewhat inconsistent. The data do show, however, that some weevils can endure a concentration of 10 percent chlorine gas in air for 1 hour, 20 percent for 20 minutes, and 50 percent for 10 minutes. That a general killing of weevils is effected by running the infested grain through a continuous treater in which it is exposed to less than 1 percent chlorine gas for about 3 minutes is shown to be highly improbable.

DISCUSSION

Although the treatment of seed with chlorine gas as practiced commercially was shown to be ineffective and impracticable, this does not necessarily remove the use of chlorine gas for seed treatment from further consideration. In some of the experiments described, chlorine gas was shown to have distinct value as a seed disinfectant when applied at the right concentration and for the proper period of time. Under these conditions it controlled bunt in wheat and covered kernel smut in sorghum satisfactorily. One drawback to its use for treating most cereal seeds is the duration of exposure necessary to bring about thorough disinfection of the seed. This makes it unadaptable to the continuous type of seed treater now in common use and makes necessary the use of some type of so-called "batch" treater in which the seed could remain exposed to the gas for the proper period of time. This method of treatment, however, is slow and more or less cumbersome and would not find favor with growers, seed dealers, and others interested in treating seed.

Chlorine gas probably could be more successfully adapted to the treatment of those kinds of seed small quantities of which will plant large acreages. It is possible that the tiny seeds of certain truck, forage, or other crops could be more profitably and easily treated with chlorine gas than with other disinfectants now in use. Investigations along this line might yield results of interest.

The outstanding objections to the use of chlorine as a seed disinfectant especially for cereals are: (1) It is disagreeable to work with, (2) it appears to have a narrow margin of safety, (3) it provides no protection to the seed against soil-borne organisms, and (4) its application calls for a type of equipment different from that now in common use for applying seed disinfectants.

SUMMARY

Chlorine gas has been advocated and, to a limited extent, employed commercially as a seed disinfectant. A patent on the use of chlorine gas as a seed disinfectant and on an apparatus for its application to seed was applied for in 1937. Observations made on this apparatus while in operation showed that treatment consisted of exposing grain to a concentration of less than 0.5-percent chlorine gas by volume for about 3 minutes. Several test lots of smutty seed of wheat, oats, and barley were thus treated in this apparatus and sown on the Arlington Experiment Farm, Arlington, Va., and in no case was improvement in emergence or stand observed nor was satisfactory smut control obtained.

In laboratory experiments, exposure of artificially smutted seed to 50- and 100-percent chlorine gas for 5 minutes failed to kill all the surface-borne smut spores.

Under certain conditions, exposure to 1-percent chlorine for 2 hours completely inhibited smut spore germination.

Bunt in wheat was adequately controlled by exposing the seed for 2 hours to chlorine concentrations of 3 to 9 percent, and covered kernel smut of sorghum was controlled by exposure to 10-percent chlorine for 1 hour without pronounced seed injury. Smuts in oats and barley were not appreciably affected irrespective of the concentration of gas or period of exposure employed.

To secure satisfactory killing of surface-borne smut spores without seed injury, the volume of gas should not be less than 20 percent or greater than 40 percent of the net volume of seed, the time of exposure should be from 1 to 2 hours, and the concentration of gas should be from 3 to 9 percent by volume.

Some adult grain weevils survived after exposure to 10-percent chlorine gas for 1 hour and to 50-percent for 10 minutes.

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